

The University of Chicago

EFFECTS OF PHOTOPERIOD ON
MICROSPOROGENESIS IN
BILOXI SOYBEAN

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

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EFFECTS OF PHOTOPERIOD ON MICROSPOROGENESIS IN BILOXI SOYBEAN

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 545

CHESTER S. NIELSEN

(WITH NINETEEN FIGURES)

Introduction

In the early work on photoperiodic response of many plants it was found that when they were returned to photoperiodic cycles not conducive to flowering, they frequently abscised their flower primordia and became vegetative (3). In other instances the flowers tended to be sterile (8). *Xanthium pennsylvanicum*, which will initiate floral primordia and produce macroscopic flowers following only one photoinductive cycle, was found by NAYLOR (6) to produce inflorescences that differed markedly in both rate and nature of development according to the number of photoinductive cycles given.

NEIDLE's work with *Xanthium* (7) indicated that both nitrogen supply and photoperiodic treatment were involved in the production of viable pollen.

GARNER and ALLARD (3, 4) found that when Biloxi soybeans were maintained on photoperiodic cycles of slightly less than 13 hours of light and 11 hours of darkness, flower primordia developed into flowers in which pollination took place, and fruits and seeds developed. If Biloxi soybeans were maintained on photoperiodic cycles of 8 hours of light and 16 hours of darkness, however, the primordia developed into small, often minute, flowers. Such flowers seldom formed full-sized pods, and few seeds were produced. In contrast with this, in plants held for a few days at the critical

period or slightly below it until flower primordia were initiated and then transferred to a photoperiod longer than the critical, a few of the primordia continued to develop into flowers much larger than those on plants maintained at or below the critical. Development of carpels on such plants was slow and these often contained no seeds.

These studies suggest that some type of cytological disturbance might be associated with the length of the photoperiod the plants were subjected to, subsequent to photoperiodic induction. The present work deals with this and was undertaken to determine the effect of various light and dark treatments on microsporogenesis in Biloxi soybeans which had previously received different numbers of photoinductive cycles.

Procedure

One of the experiments reported here was conducted in the spring and summer of 1939, while the other two were conducted during the spring and autumn of 1941. In all three experiments environmental conditions up to the time of treatment were made as nearly identical as possible. Seeds were planted in 4½-inch clay pots filled with a rich loam soil. The seeds were germinated on well-lighted greenhouse benches, where supplementary illumination from Mazda filament lamps extended the length of the photo-

period. This light, of approximately 75 foot-candles at the leaf surface, was supplied from sunset until 2:00 A.M., thereby affording a photoperiod of about 21 hours out of 24. This photoperiod of 21 hours of light and 3 hours of darkness was supplied from the time the first trifoliate leaf was beginning to expand until treatment was begun.

Just before treatment, the plants were selected for uniformity in size and vigor and divided into several lots, each of which (except the controls) received a specific number of photoinductive cycles (table 1). These photoinductive cycles of 8 hours of light and 16 hours of darkness were provided by drawing a double-thickness black sateen cloth over the light framework arranged over the bench at 4:00 P.M. and removing it at 8:00 A.M.

In the first and second experiments, each lot of plants, except the controls, received from three to nine photoinductive cycles before being returned to long photoperiod conditions of 21 hours of light and 3 hours of darkness. In the third experiment different lots of plants received from two to ten photoinductive cycles before being returned to long photoperiod conditions, which in this case were 16 hours of light and 8 hours of darkness.

Three types of controls were employed. One group of plants remained on long photoperiod until harvested. None of these ever flowered. The second group was maintained on natural photoperiod until harvested. The third group remained on short photoperiod until harvested.

Subsequent to photoinductive treatment, collections were made at regular intervals from each lot in each of the experiments, according to the procedure outlined in table 1. In each of the experi-

ments collections were begun at progressively later dates. Thus in the first experiment collections were begun as early as 1 day after photoinductive treatment and were continued for more than 1 month. In the second experiment collections were begun 22 days after treatment had been given and continued for 2 months. Collections in the third experiment started 26 days after photoperiodic treatments were given and continued for more than $2\frac{1}{2}$ months.

To obtain material for cytological study, the main axis of the plant was cut into short segments, and each node bearing floral buds was placed in Sax's modification of Navashin's solution. The material was washed in tap water and run up in ethyl-tertiary butyl alcohol to paraffin. Sections were cut at 8-10 μ , stained with Heidenhain's iron-alum haematoxylin, and counterstained with orange G in clove oil.

Results

None of the plants in any of the experiments—with the exception of the control lots which received natural photoperiod and those which were maintained on photoinductive cycles—had opened their flowers by the end of any collecting period. In every instance the time permitted the plants to mature exceeded the time recorded by BORTHWICK and PARKER (1) as being sufficient for maturation following a specific number of photoinductive cycles. This difference in time required for blooming is probably attributable to the difference in length of photoperiod used following the photoinductive cycles, or to a difference in light intensity or quality at Beltsville, Maryland, and Chicago, Illinois.

Examination of the axillary buds of the control plants showed that the flower primordia first appear as enlarg-

ing protuberances in the axils of bracts along the axis. The stamen primordia appear while the petal primordia are still small. The latter grow very slowly until the microsporocytes, characterized by dense cytoplasm and a relatively large nucleus (as contrasted with somatic cells), are differentiated in the anthers (fig. 1).

otic metaphases and anaphases show the haploid number of chromosomes to be 20, which is in agreement with the reports of FUKUDA (2) and VEATCH (9). Anaphasic separation of the bivalent chromosomes follows quickly (figs. 5, 6), resulting in the formation of two nuclei which pass into an interphase of short duration (fig. 7). The second meiotic di-

TABLE 1

LOT NO.	AGE IN CYCLES OF LONG PHOTOPERIOD AT TIME OF TREATMENT			NO. OF CYCLES OF SHORT PHOTOPERIOD			NO. OF CYCLES OF LONG PHOTOPERIOD FOLLOWING INDUCTION*						
	EX- PERI- MENT I	EX- PERI- MENT II	EX- PERI- MENT III	EX- PERI- MENT I	EX- PERI- MENT II	EX- PERI- MENT III	EXPERIMENT I		EXPERIMENT II		EXPERIMENT III		
							FIRST PLANT	LAST PLANT	FIRST PLANT	LAST PLANT	FIRST PLANT	LAST PLANT	
1A.....	On cycles of long photoperiod until harvested; entirely vegetative												
1B.....	27	Control plants, on cycles of natural photoperiod, from 14 to 71 cycles until harvested											
2.....			25			2						59	80
3.....	27	23	25	3	3	3	9	26	29	61	58	79	
4.....	27	22	25	4	4	4	8	31	29	61	52	73	
5.....	28	22	25	5	5	5	7	35	30	52	43	64	
6.....	29	22	25	6	6	6	5	33	29	51	40	61	
7.....	32	22	25	7	7	7	1	30	26	47	35	56	
8.....	33	22	25	8	8	8	1	29	25	46	28	50	
9.....	34	24	25	9	9	9	1	36	22	43	27	49	
10.....			25			10					26	48	
11.....	27	On cycles of short photoperiod, from 13 to 30 cycles until harvested											

* Plants from each lot harvested progressively, so that material in various stages of development would be available. Number of cycles of long photoperiod which first and last plants of lot received after photoinduction is indicated.

Growth of the microsporocyte and its nucleus soon occurs. The characteristic reticulum of the metabolic nucleus is transformed into the slender thread stage of the leptotene. The threads appear as numerous elements, rather uniformly distributed throughout the nucleus. Nuclear diameter increases markedly as the synaptic pairing of the zygotene proceeds to the pachytene stage (fig. 2). Following diplotene and diakinesis, the bivalent chromosomes are next arranged on the equator of the spindle (figs. 3, 4). Repeated counts made at the first mei-

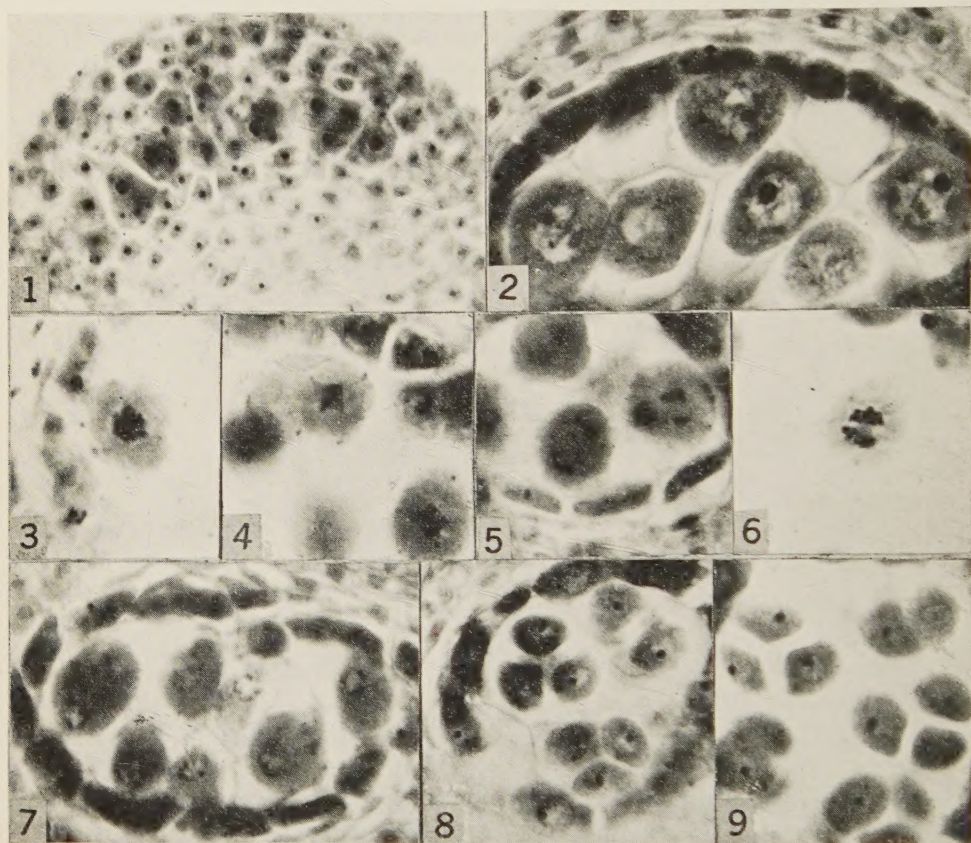
vision produces the four-nucleate stage, which is soon followed by cleavage to form the tetrahedrally arranged quartet of microspores (fig. 8). Disintegration of the microsporocyte wall takes place as the microspores within it round up and their walls thicken (figs. 9, 10).

Examination of buds from the photoperiodically treated plants showed that flower primordia had been initiated and had developed essentially as in control plants, up to the differentiation of microsporocytes. Abnormalities in microsporangogenesis now begin to appear, the time

and degree depending in part upon the number of photoinductive cycles which the various lots of plants had received.

The first observable abnormality in the microsporocytes is a vacuolation of the cytoplasm. The vacuoles increase in size as the cells enlarge, and there ap-

haematoxylin (fig. 11). On the other hand, enlargement and vacuolation may continue, resulting in large cells with little cytoplasm and small nuclei. Successive stages in this process are shown in figures 12 and 13. The last stages are represented by shriveled anthers, the

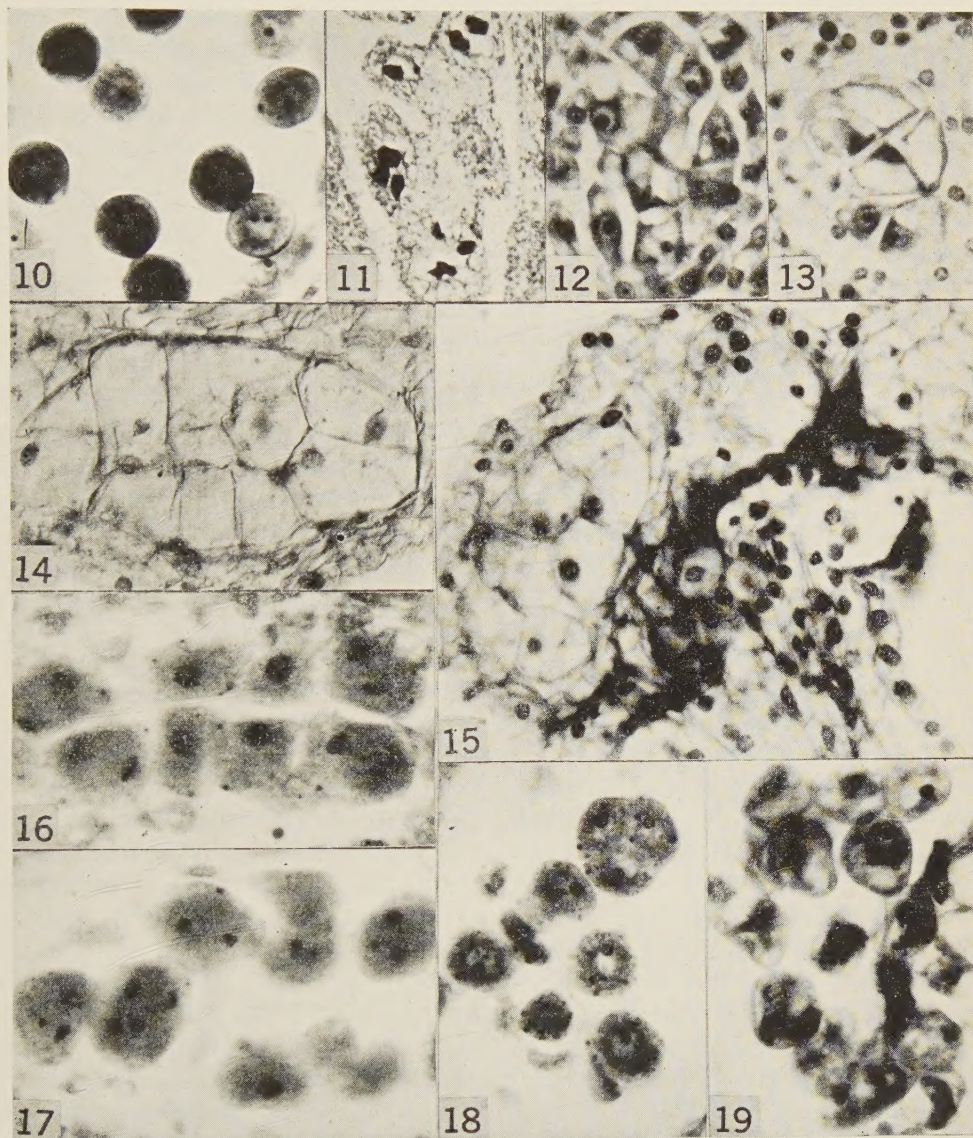


FIGS. 1-9.—From control plants of lot 1B: Fig. 1, young microsporocytes; fig. 2, pachytene; figs. 3, 4, metaphase I; figs. 5, 6, anaphase I; fig. 7, interkinesis; figs. 8, 9, quartets.

pears to be little, if any, increase in volume of the cytoplasm or of the nucleus. Cell enlargement may terminate at any point before the size characteristic of normal microsporocytes is reached. Degenerative changes may set in while enlargement is proceeding, often resulting in irregular masses of disintegrating sporogenous tissue which stains intensely with

locules of which contain cells with scanty cytoplasm and whose nuclei are either small and degenerated or occasionally entirely lacking (fig. 14). Shortly afterward the anther wall begins to break down, and eventually the entire stamen collapses (fig. 15).

In the lots receiving fewer than six cycles of short photoperiod, degenera-



FIGS. 10-19.—Fig. 10, normal microspores from plant of lot 1B. Fig. 11, shriveled anther containing degenerated microsporocytes, lot 6. Figs. 12, 13, microsporocytes showing vacuolation and early degeneration, lot 8. Fig. 14, locule of anther with enlarged microsporocytes containing little cytoplasm and unenlarged nuclei, lot 6. Fig. 15, stamen showing early stages of degeneration of wall cells, lot 8. Fig. 16, anaphase I, lot 8. Fig. 17, anaphase II, nuclei irregular and abnormal, lot 8. Figs. 18, 19, microspores showing various stages of degeneration, lot 8.

tion of the microsporocytes at early stages of microsporogenesis is characteristic. In only one instance was morphologically normal microspore development observed. A few plants given six cycles of short photoperiod have shown development beyond the microsporocyte stage. The meiotic process may proceed apparently normally through the first meiotic division (fig. 16) and as far as the anaphases of the second (fig. 17). In many cells there is frequently evidence of chromosomal degeneration at these stages, with consequent failure of telophasic reorganization. Some cells in which an apparently normal telophasic reconstruction occurs may undergo premature cleavage, followed by collapse of the resulting abnormal microspores. Normal meiosis does occur in the microsporocytes of some anthers of plants which had received six or more photoinductive cycles, and tetrads are formed which appear capable of developing into functional microspores (fig. 19). A high percentage of the microspores in these plants undergo degeneration, however, even at these advanced stages of development.

Differences in degenerative changes between experiments I and II, which had received postinductive photoperiods of 21 hours, as compared with those of experiment III, receiving 16 hours, are quantitative rather than qualitative, in that abnormalities of the same type occur in all but are more intense in the first two. In all the experiments, regardless of the character of the postinductive photoperiods, less than six inductive cycles of short photoperiod result in suppression of microsporogenesis prior to the formation of microspores. With six or more inductive cycles, apparently normal as well as abnormal pollen was produced, with an increase in

percentage of the former as the number of inductive cycles approaches ten. Nevertheless, in the lot receiving ten cycles of short photoperiod, although degeneration in earlier stages was less, disintegration of microspores and the presence of nonviable pollen is still apparent.

Discussion

The degenerative changes observed during microsporogenesis in Biloxi soybean which had been given fewer than six photoinductive cycles and then returned to a postinductive photoperiod of 16 or 21 hours have been found to be similar, in many respects, to the effects of carbohydrate deficiency in the tomato as described by HOWLETT (5). He found that severe carbohydrate deficiency resulted in failure of the nuclei of sporogenous cells to reach meiotic divisions. The walls of these cells collapsed and the protoplasts degenerated before the prophase were initiated. This occurred concurrently with retardation in development of the entire flower as a result of limitation of carbohydrates.

Early degeneration of the sporogenous tissues likewise occurred in all the groups of photoperiodically induced soybean plants used in these experiments, but it was only in those groups receiving five or fewer photoinductive cycles that development failed to proceed beyond the early prophase stages of meiosis. And even in these groups the perianth segments were not noticeably retarded in development up to this time.

In the groups given six to ten photoinductive cycles, degeneration at later stages in meiosis was frequent. In some anthers, microsporocyte enlargement occurred regularly; but during its progress increasing vacuolation of the cytoplasm, accompanied with nuclear disintegration, resulted in the production of shriveled

anthers containing disorganized, densely staining masses of sporogenous cells. In other anthers the sporocytes appeared to undergo an apparently normal meiosis and produced four groups of chromosomes, each inclosed by a nuclear membrane. In those plants given seven or eight photoinductive cycles, degeneration frequently occurred before the four groups of chromosomes were reorganized as resting nuclei and before cleavage into microspores took place. Even when resting nuclei were organized, as was often observed in plants given nine or ten photoinductive cycles, degeneration of the microspores might still occur immediately following cleavage. Morphologically normal microspores were found, however, in many of the plants which had received eight to ten photoinductive cycles, despite the high percentage of degeneration in earlier stages of meiosis.

As in carbohydrate-deficient tomato plants, where the degree of degeneration was directly proportional to the severity of the deficiency and to the level of development at which it is operative, the number of photoinductive cycles in the soybean influences similarly the degree and stage to which normal development of microsporocytes may proceed. The length of the postinductive photoperiod appears also to be an important factor affecting the degree of sterility in the plants of these experiments, when they are compared with the results obtained by BORTHWICK and PARKER, who used natural photoperiod following the photoinductive treatments.

Summary

1. Three experimental series of Biloxi soybeans were given photoinductive treatments of from two to ten cycles, consisting of 8 hours of natural daylight followed by 16 hours of darkness. After

induction, series I and II were placed on cycles of long photoperiod, consisting of 21 hours of light and 3 hours of darkness, and series III on cycles of 16 hours of light and 8 hours of darkness.

2. Three types of controls were employed. One group of plants was kept on long photoperiod. None of these flowered. A second group was kept on natural photoperiod and a third on short photoperiod. These two groups flowered.

3. Floral buds from controls and from the three experimental series were collected progressively in order to obtain various stages in development. In the second and third groups of controls, floral development was normal and meiosis occurred as has been described by previous workers. In all the experimental groups the floral structures developed normally until the differentiation of sporocytes had occurred in the anthers. Following this, various abnormalities in meiosis were observed.

4. In plants given five or fewer photoinductive cycles, the sporocytes might begin to enlarge, accompanied by marked vacuolation of the cytoplasm and ultimate disintegration of their nuclei. Only a few of the sporocytes ever reached the metaphase stage of meiosis I.

5. In plants receiving six or more photoinductive cycles of short photoperiod, degeneration of microsporocytes in some anthers may occur as already described, but in others the sporocytes undergo apparently normal meiosis, and four groups of chromosomes are formed. In some instances degenerative changes occur at this point before cleavage into microspores takes place; in others cleavage occurs followed by degeneration before development of normal microspores has been accomplished; while in a very small percentage of instances apparently normal microspores are formed. Even

those plants receiving as many as ten photoinductive cycles of short photoperiod showed a high percentage of degenerated microspores.

6. The long postinductive photoperiod employed in these experiments appears to have played a significant role in bringing about the degenerative changes ob-

served and in suppressing the development of mature flowers.

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